2693-Pos Board B679
Conformational Characterization of Tachykinin Neuropeptides: Role of the Polyproline II Structure
Tzvetana R. Lazarova, Arash Foroutan, Francesc Sepulcre, Esteve Padrós.
Substance P (SP), Neurokinin A (NKA) and Scyliorhinin I (Scyl) peptides belong to the Tachykinin family and are agonists for Neurokinin1 (NK1) GPCR receptor with different potent activities. These Tachykinins are involved in several physiological processes and some neurodegenerative disorders, what make them relevant therapeutically agents. The characterization of the active peptide conformations of the Tachykinins is essential for the elucidation of the mechanism of action and for the design of drugs. To address the molecular basis for the peptide recognition by the NK1 receptor we studied the conformation of SP, NKA and Scyl in solution and membrane-mimic environments: micelles and liposomes, by using CD and FTIR spectroscopies. The analysis of CD data revealed that the three peptides form a partially α helical structure in the presence of the negatively charged micelles and vesicles, but not in zwitterionic DMPC. By performing CD spectra at increasing temperatures we demonstrated that in an aqueous environment SP, NKA and Scyl form extended polyproline II (PPII) helical structure. The same structure was found in the membrane mimics, which do not induce formation of α helical conformation of the peptides. FTIR experiments performed in D_2O support the presence of PPII conformation. Further we questioned whether the formation of PPII needs peptide binding to the membrane or it is a peptide intrinsic property, by using fluorescence techniques. We propose that the peptide-membrane interaction is a two-step process involving first electrostatic interactions through PPII structure, followed by the folding and insertion of the alpha-helical segment.

2694-Pos Board B680
Bicelle-Bound Solid-State NMR Structural Studies and Membrane-Permeabilizing Activities of Piscidin 1 and Piscidin 3: Implications for Mode of Antimicrobial Action
Matthew K. Baxter, Jason A. McGavin, Nina B. Kraus, Anna A. De Angelis, Jolita Seckute, Caitlin Burzynski, Daryl M. Berke, Nedzada Smajic, Linda K. Nicholson, Stanley J. Opella, Myriam Cotten.
Bicelles represent a novel preparation of hydrated lipid bilayers, which can be used to study membrane-associated proteins under physiologically-relevant conditions. Large bicelles can be oriented within a magnetic field, enabling the determination of high-resolution peptide structures and angles of insertion within a lipid membrane via solid-state Nuclear Magnetic Resonance (NMR). Piscidin, an amphipathic, antimicrobial peptide found in hybrid striped bass, plays a major role in host defense. It is effective against a wide range of pathogens, including methicillin-resistant Staphylococcus aureus and HIV-1. The peptide is known to have an alpha-helical conformation when bound to anionic lipid membranes that mimic the surface of bacterial membranes.
We have investigated the use of bicelles in the study of piscidin. 15N NMR spectra show that piscidin has been successfully aligned in magnetically oriented bicelles. 31P NMR studies, which show that piscidin disrupts bicelle-forming lipids, have helped us better understand its mode of action. To complement these backbone solid-state NMR studies of piscidin, we have used fluorescent-dye leakage experiments with various phospholipids and have performed solution NMR to determine the charge state of the histidine side chains in the presence of micelles and investigate their possible role in mediating important peptide-lipid interactions. The long term goal of this project is to improve our understanding of structure function relationships in an interesting family of antimicrobial peptides. This knowledge could be used to design potent antimicrobial pharmaceuticals that minimize bacterial resistance.

2695-Pos Board B681
Atomic-Resolution Three Dimensional Structures and Membrane Locations of Antimicrobial Piscidin 1 and Piscidin 3 in Aligned Lipid Bilayers: A Solid-State NMR and Molecular Dynamics Investigation
Myriam Cotten, William E. Wieczorek, Mukesh Sharma, Milton Truong, Breanna S. Vollmar, Eric D. Gordon, Richard M. Venable, Richard W. Pastor, Riqiang Fu.
Piscidin, an amphipathic cationic antimicrobial peptide (AMP) active against a broad range of pathogens including multidrug-resistant bacteria and HIV-1, belongs to a large family of vital host-defense peptides that interact, at least initially, with negatively-charged microbial membranes in order to perform their function. While two piscidin isoforms, piscidin 1 (p1) and piscidin 3 (p3), are highly homologous, they display unequal antimicrobial and hemolytic effects. As a way to identify factors optimizing specific molecular interactions directly related to their mode of membrane activity, we have investigated p1 and p3 bound to lipid membranes that mimic bacterial membranes.
Previously, we used solid-state NMR on 15N-labeled peptides to demonstrate that membrane-bound p1 and p3 adopt an alpha-helical structure and lie in the plane of hydrated lipid bilayers where they experience fast dynamics. Our recent analysis of two-dimensional solid-state NMR data has lead to the first atomic resolution three-dimensional backbone structures of p1 and p3 bound to aligned lipid bilayers. Structural calculations based on the NMR data and molecular dynamics simulations have been performed to yield a refined structure and membrane location for each peptide.
We will explain how our atomic-level investigation of the structure, dynamics, and bilayer location of piscidin provides new insights into its mode of action and therefore allows us better to understand how AMPs disrupt bacterial membranes and induce cell death. The long term goal is to derive common principles that could facilitate the design of pharmaceuticals with enhanced antibacterial activity and lower toxicity on mammalian cells.

2696-Pos Board B682
The Importance of the Proline Hinge in the Action of Histone-Derived Antimicrobial Peptides
Kathryn E. Pavia, Sara A. Spinella, Kathy J. Chen, Donald E. Elmore.
Antimicrobial peptides (AMPs) are short, polycationic proteins capable of killing a wide variety of bacterial species through a number of different mechanisms. The ability to effectively engineer potent, cell-penetrating AMPs would maximize the full potential of these molecules. Buforin II (BF2), a cell-penetrating histone-derived antimicrobial peptide (HDAP), served as a model for the design of three novel histone-derived peptides, DesHDAPs1-3. BF2 is has a C-terminal α-helix that is broken by a proline hinge. Because this proline hinge determines, in part, BF2's ability to translocate into and kill bacterial cells, DesHDAPs1-3 were designed to also contain a helix-breaking proline residue. To determine whether this structural feature plays the same role in the designed peptides, the activity of proline to alanine mutants of each designed peptide were tested against a variety of bacterial species. As expected, circular dichroism measurements indicate that the proline to alanine mutation increases the α-helical character of BF2 and all designed peptides. For both BF2 and DesHDAP1, proline to alanine mutants show decreased antimicrobial activity against all species tested. In contrast, proline to alanine mutations in both DesHDAP2 and DesHDAP3 either do not affect or slightly increase the observed antimicrobial activity. This suggests that α-helicity is a poor predictor of antimicrobial activity for this family of HDAPs, and that the proline residue may play a different role in DesHDAP2 and DesHDAP3 than it does in BF2 and DesHDAP1. In order to explain these trends, we have further characterized the translocation and membrane permeabilization properties of the proline to alanine mutations. As well, we have used molecular dynamics simulations to explore the structure of the membrane bound peptides.

2697-Pos Board B683
Structure of Peptide-Induced Transmembrane Pore Determined by Anomalous X-Ray Diffraction
Ming-Tao Lee, Shiuan-Shiaou Wu, Wei-Yu Lin, Yi-Ting Sun, Wei-Chin Hung.
We determined the structure of the melittin-induced transmembrane pore by X-ray diffraction. The multibilayer sample on substrate was prepared in full hydration. The peptide-to-lipid ratio, P/L, of the melittin-lipid mixtures were in the condition where pores were present, as established previously by neutron in-plane scattering in correlation with oriented circular dichroism. At low hydration levels, the interbilayer distance shortened and caused the membrane pores to become long-ranged correlated and form a periodically ordered lattice of rhombohedral symmetry. Here we used the multiwavelength anomalous dispersion (MAD) method to solve the phase problem for a rhombohedral phase of a phospholipid with brominated chains and performed multiwavelength anomalous diffraction at the bromine K edge. The X-ray light source in BL23A beam line of NSRRC and home-made humidity-temperature controlled chamber will be applied in the measurements. We found the melittin-induced pores were at least partially framed by a lipid monolayer. Evidence suggests that the pore structure is of the toroid type different from the barrel-stave type induced by alamethicin.

2698-Pos Board B684
High Throughput Screen of Combinatorial Peptide Library for Gain-of-Function and Loss-of-Function Changes to Melittin
Aram J. Krauson, William C. Wimley.
Melittin, the main peptide component of European Honey Bee venom, is an amphipathic, 26-amino acid peptide that lyses bacterial and mammalian cells by forming transmembrane pores. Our research focuses on the various mechanisms of peptide permeation of membranes. We have designed orthogonal, fluorescence-based assays to characterize long-lived pore-forming peptides such as melittin. In these assays, peptides are incubated overnight with vesicles containing dye-labeled lipids and entrapped terbium. In the first measurement, the sum of the lytic activity is determined by measuring the terbium released from the vesicles. In the second measurement, we add

a non membrane-permeable quencher of dye-labeled lipids. Using these assays we have observed a significant difference between melittin and other lytic peptides, such as alamethicin. Even at very high lipid concentrations (peptide:lipid < 1:2000) alamethicin forms long-lived pores, which release ~100% of entrapped contents and give the quencher 100% access to the inside of a vesicle after overnight incubation. Melittin's activity diminishes significantly at lipid concentrations larger than P:L = 1:500. To learn what factors modulate melittin's activity, we have designed a 7,776-member, melittin-based combinatorial peptide library in which we vary critical residues in its natural sequence. We also incorporate self-associating motifs that are found in alpha-helical membrane proteins. Library members were screened using the orthogonal assays at very high and very low stringency. Selected positives from the highly stringent assay (i.e. gain in activity sequences) show a high frequency of alanine substitutions at specific polar and basic residues. Selected negatives from the low-stringency assay (i.e. loss of activity sequences) show two key nonpolar-to-glycine replacements as well as a substitution of the proline residue. Selected peptides from both screens have been contrasted to melittin's activity by using biophysical techniques, antimicrobial and hemolytic assays.

2699-Pos Board B685

Inhibition of Melittin Activity by Cholesterol, Unsaturated Lipid, and Negatively Charged Lipids Studied by Molecular Dynamics Simulation

Jian Dai, Mohammad Alwarawrah, Juyang Huang.

Melittin is shown to cause membrane lyses. However, its lytic activity depends on the lipid composition of the membrane. Experiments have shown that the presence of some lipid components in the membrane can inhibit melittin's lytic power against the membrane. For the sake of atomistic details, molecular dynamics simulations was used to investigate the inhibition of melittin activity by cholesterol, unsaturated phospholipid (POPE), and negatively charged phospholipid (POPS). A pure DPPC lipid bilayer with melittin was simulated as a control, and significant disturbance of the bilayer by melittin was found. The order parameter of DPPC changed dramatically and a large curvature was observed in one of the membrane leaflets, probably leading to a future membrane rupture. The DPPC bilayer with cholesterol showed strong resistance to melittin: Melittin can hardly bind to the membrane surface. In the simulation with unsaturated lipid, melittin can only bind either its N or C terminal region to the bilayer, but the interaction between the body of melittin and the lipids is weak. Melittin binds strongly to negatively charged lipids; however, it cannot induce membrane curvature nor disruption. In above three simulations, C termini of melittin were observed to be able to bind to the membrane more easily than N termini. A deep and stable adsorption of melittin to the membrane requires the binding of both N and C-terminal regions to the membrabe. The tendency of melittin to aggregate was observed in all simulations, especially in the simulation containing cholesterol. This study provides insight into the possible mechanisms of the inhibition of melittin's lytic activity by different membrane components.

2700-Pos Board B686

Melittin vs E.coli: Insights from Molecular Dynamics Simulations

Syma Khalid, Thomas J. Piggot.

The interactions of antimicrobial peptides with eukaryotic cell membranes have been well-studied by experimental and computational methods. However, the molecular-level interactions of these peptides with Gram-negative bacteria such as E.coli are more difficult to study, largely due to the complex nature of the asymmetric outer membrane of Gram-negative bacteria. While cell lysis is thought to proceed via destruction of the inner membrane, it is the outer membrane that is the first barrier encountered by antimicrobial peptides as they attempt to reach their target site.

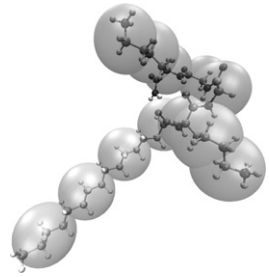
In the present work, we employ atomistic molecular dynamics simulations to study the interaction of melittin, the major component of honey bee toxin, with detailed models of the E.coli outer membrane. Experimental data (ref), has indicated that upon interaction, melittin induces reorganisation of the lipopolysaccharide (LPS) component of the bacterial outer membrane. We have simulated multiple copies of melittin in lipid bilayers composed of varying depths of LPS in the outer leaflet, and realistic mixtures of phospholipids in the inner leaflet. Our simulations reveal details of the melittin-outer membrane recognition process and the structural stability and dynamics of melittin as it interacts with the outer membrane. Furthermore, our simulations provide specific, molecular-level details of the interaction of melittin with the E.coli outer membrane that may aid the future design of novel antimicrobial agents.

2701-Pos Board B687

Characterization of Potent Antimicrobial Lipopeptide via All-Atom and Coarse-Grained Molecular Dynamics

Joshua N. Horn, Jesse Sengillo, Alan Grossfield.

The prevalence of antibiotic resistant pathogens is a major medical concern, prompting increased interest in the development of novel antimicrobial compounds. To characterize a potent, synthetic lipopeptide, C16-KGGK, microsecond time-scale all-atom simulations with the CHARMM forcefield are utilized. This lipopeptide targets the bacterial membrane, but the binding and reorganization processes are extremely slow. To increase simulation speed, supplemental coarse-grained simulations with the MARTINI forcefield are used. This combination provides insights into the structure, dynamics, binding and mechanism of antimicrobial action.



2702-Pos Board B688

Electrostatic Interactions between Antimicrobial Peptides and Anionic Membranes: Insights from an Implicit Membrane Model

Yi He, Lidia Prieto, Themis Lazaridis.

Electrostatic interactions between antimicrobial peptides (AMPs) and anionic bacterial plasma membranes are crucial for their activity and selectivity. In previous implicit models of anionic membranes, the effects of dipole potential and the presence of pores were not included. In this work, we studied the electrostatic interactions of AMPs with the anionic lipids using a membrane model where double layers of charges are used to represent the head group dipole. The electrostatic interactions between AMPs and the anionic membranes and their influence on pore formation are discussed considering the contributions from the dipole potential and the surface potential. We found that the dipole potential had a strong effect on peptide binding and insertion: their location and orientation changed as a function of the strength and the sign of dipole potential. The surface potential, which is caused by the excess charge of anionic lipids however, has influence only on binding. Alamethicin, which is known to form barrel-stave pores, favors binding to cylindrical shaped pores. Melittin and magainin, on the other hand, strongly favors binding to parabola shaped pores, in agreement with the experiments. For these two peptides, increased anionic lipid content enhances this trend while increased dipole potential has the reverse effect.

2703-Pos Board B689

Molecular Dynamics Simulations of Influenza Fusion Peptide: A Correlation between Flexibility and Fusogenicity

Sébastien Légaré, Patrick Lagüe.

A flu infection starts with the entry of the influenza virus into an host cell. Entry requires endocytosis of the virus by the host cell and subsequent fusion between the viral and endosomal phospholipid membranes. This important membrane fusion step is catalysed by the influenza fusion peptide whose action mode remains unsung. Detailed knowledge on the fusion peptide is essential for the development of therapeutics as well as to understand many biological processes since membrane fusion is ubiquitous to life.

Previous experiments on fusion peptides from various influenza strains and their respective mutants aimed to unveil a structure-function relationship without general agreement. The peptides were shown to adopt a helix-hinge-helix motif essential for fusogenicity, but results diverged on the hinge region which strongly impacts on the peptide structure. The hinge region was sometimes shown as flexible (Dubovskii, Prot. Science 9:786), as a fixed kink (Han, Nat. Struc. Biol. 8:715) or as a tight hairpin (Lorieau, PNAS 107:11341). Similar disagreement occurred in modelling studies (Jang, Proteins 72:299 ; Li, J. Phys. Chem. B 114:8799 ; Panahi, J. Phys. Chem. B 114:1407).

In this work, the structure-function relationship of the fusion peptide from type three hemagglutinin and two mutants (F9A and W14A) were studied from extensive explicit solvent molecular dynamics simulations. Our results show that the hinge region of the fusion peptide is flexible and allows it to be in an equilibrium between kinked and helical conformations. The two mutants also show a different flexibility than the wild-type. Moreover, a correlation between peptide flexibility, lipid protrusion (proposed as the membrane fusion catalysis mechanism (Kasson, PLoS Comput. Biol. 6:e1000829)) and fusogenicity reported in the literature was observed.